REMARKS

This Reply is responsive to the Office Action dated April 25, 2008. Claims 68-174 are currently pending in the application. Claims 68-106, 169 and 170 are withdrawn, and claims 107-168 and 171-174 are under examination. Entry of the remarks submitted herein and reconsideration of the claimed subject matter is respectfully requested.

I. Rejection under 35 USC §103

The Examiner has maintained the rejection of claims 107-168 and 171-174 under 35 U.S.C. §103(a) as being unpatentable over Werther *et al.* (US 5,929,040), Fire *et al.* (US 6,506,559), Heifetz *et al.* (WO 99/61631), Calabretta *et al.* (US 5,734,039) and Thompson *et al.* (US 6,146,886) for the same reasons set forth in the Office Actions dated March 8, 2007 and September 7, 2007. According to the Office Action, Werther *et al.* allegedly teaches a multivalent antisense molecule but does not disclose the use of double stranded RNA sequences or the expression of double stranded RNA sequences from a vector. Calabretta *et al.* allegedly describes a composition comprising two antisense molecules directed to one or more target genes and suggests that the antisense molecules might be expressed from a single vector using two different promoters. However, the Examiner acknowledges that Calabretta *et al.* does not teach the use or expression of double stranded RNA sequences and does not actually demonstrate expression of multiple RNA sequences. Nevertheless, the Examiner believes it would have been obvious to one of ordinary skill in the art at the time the invention was made to substitute double stranded RNA as allegedly disclosed in Fire *et al.* and Heifetz *et al.* as an alternative for the antisense sequences in the constructs of Werther and Calabretta and

express these molecules in a vector as allegedly also disclosed in Fire *et al.* and Heifetz *et al.* Thompson is relied upon for teaching expression of therapeutic RNAs including ribozymes and antisense RNAs using a RNA pol III promoter. Applicants respectfully traverse the rejection.

The currently pending claims are directed to partially double stranded RNA molecules comprising two or more different double stranded RNA sequences that are substantially homologous and complementary to two or more sequences of at least one target mammalian gene or mammalian pathogen gene, vectors expressing such RNA molecules, and expression vectors encoding multiple double stranded RNA sequences that are expressed from different promoters. Applicants again assert that none of the cited references alone or in combination render the subject matter of the instant claims obvious.

As extensively detailed in Applicants' response of February 6, 2008, Fire is not enabled for inhibiting gene expression in mammalian cells with double stranded RNA. The Examiner alleges that the Fire patent is "presumed to be enabled" because "thousands of post-filing art references have repeatedly shown that the methods of Fire *et al.* work in human cells." See Office Action, page 4. The Examiner goes on to say that Fire *et al.* received the Nobel Prize for their discovery based upon "the implications of its use in humans." The Examiner does not provide any evidence for these statements and in fact, overlooks the references cited by Applicants in their response of February 6, 2008 which show that the methods disclosed by Fire do not work in mammalian cells. Furthermore, Applicants note that a U.S. patent is presumed to be valid for all that it claims. Since Fire does not claim a method of inhibiting gene expression in mammalian

cells, it is not presumed to be enabled for such a method. In fact, the prosecution file history of the Fire patent and its related applications would suggest the contrary.

In addition, the disclosure of a prior art reference is enabling if "the public was in possession of the claimed invention before the date of the invention." M.P.E.P. § 2101.01. As evidenced by the several references cited in Applicants' previous two responses and the declaration by Dr. McCallus submitted with the last response, it was clear that the skilled artisan could not use the methods disclosed by Fire directly to inhibit gene expression in mammalian cells prior to the instant invention because of the presence of the PKR response in these cells. Indeed, Fire himself expressed doubt as to whether his methods would work in mammalian cells because of the non-specific response that these cells exhibited to double stranded RNA (*e.g.* PKR response). See Montgomery and Fire (1998) Trends in Genetics, Vol. 17: 255-258 (submitted as document # 415 in the IDS dated February 6, 2008). Specifically, Fire states that "Any gene-specific interference by dsRNA in PKR-proficient mammalian cells would be dependent on a transient lapse in the PKR response, or on a controlled level of dsRNA that was incapable of activating PKR." *Id.* at 258. Therefore, the methods of Fire for using dsRNA to inhibit gene expression in mammalian cells were not enabled prior to the present invention due to the problems encountered with the well known phenomenon of the PKR response in mammalian cells.

Applicants assert that a skilled artisan would not have had a reasonable expectation of success at combining the double stranded RNA molecules disclosed in Fire and Heifetz for the antisense sequences in the constructs disclosed by Werther and Calabretta as the Examiner alleges. As discussed above, Fire teaches the use of double

stranded RNA to inhibit gene expression in nematodes, but does not enable the use in mammalian cells. Heifetz teaches the use of double stranded RNA molecules in plants.

As extensively explained in Applicants' previous response, it was well known at the time of the present invention that an interferon or PKR response was induced when double stranded RNA molecules, particularly long double stranded RNA molecules, were introduced into mammalian cells. These arguments were supported by several references in the literature as well as by a declaration by Dr. McCallus, who is one of skill in the art. The Examiner has dismissed this evidence by claiming that the references which Dr. McCallus relied upon in his declaration are "references that relate to the effects of long dsRNA and the ability of the dsRNA to bind and activate protein kinase, which is entirely unrelated to the process of RNA interference." See Office Action, page 6. It is apparent from this statement that the Examiner has misunderstood the PKR response and its effect on the process of RNA interference. As documented by the references in Dr. McCallus' declaration, double-stranded RNA molecules longer than 30 base pairs bind to and activate the PKR protein kinase, which is the initial step in the PKR response. Once activated, PKR kinase triggers a signal transduction cascade that results in the phosphorylation and inactivation of the eIF2 alpha translation factor, which in turn shuts down general protein synthesis and triggers cell death. Therefore, a general arrest of protein synthesis and the induction of apoptosis would prevent any sequence specific gene silencing by an RNA interference mechanism. Contrary to the Examiner's assertion that the references cited in Dr. McCallus' declaration are irrelevant, the references rebut the Examiner's presumption that one of skill in the art would have reasonably expected success when using double stranded RNA to inhibit gene expression in mammalian cells

based on the disclosure of Fire. The references are representative of the extensive body of literature demonstrating why the RNA interference methods disclosed by Fire would not have been expected to work in mammalian cells. Thus, a skilled artisan attempting to silence specific mammalian target genes would not use the double stranded RNA molecule approach as he would expect a suppression of all protein synthesis and possible cell death as extensively described in the literature at that time.

Applicants submit that they have not merely reduced to practice the methods taught by Fire, because Fire does not disclose or suggest the experiments outlined in the Examples of the present invention. Furthermore, one of skill in the art would not have combined with a reasonable expectation of success the double stranded RNA molecules disclosed in Fire with the multivalent antisense constructs disclosed in Werther to make multitarget molecules as claimed in the present invention for use in mammals. As discussed above, a skilled artisan would not have expected the double stranded RNA molecules disclosed in Fire to be effective in silencing genes in mammalian cells because of the PKR response. The Examiner alleges that Fire sufficiently teaches one of skill in the art to carry out the invention in mammalian cells and supports her position by referring to the "voluminous" post-filing art that purportedly demonstrates that the methods of Fire work in human cells (Office Action, page 5). However, the Examiner does not cite even a single reference to support this statement. Applicants wish to remind the Examiner that the predictability of whether a modification of the prior art disclosure would have a reasonable expectation of success is to be determined as of the time when the instant invention was made. See M.P.E.P §2143.02 (III). The Examiner has not provided any evidence at the time of filing of the instant application that demonstrates

that the methods of Fire could be used in mammalian cells. The numerous post-filing references showing that the methods of Fire work in human cells that the Examiner alleges exist are not relevant if they were published after the time the present invention was made. Given that the PKR response was known to be an obstacle for RNA interference at the time of the present invention and the fact that artisans in the field could not observe sequence specific silencing in mammalian cells with double stranded RNA (see Caplen et al., 2001; cited in previous response) suggest that a skilled artisan would not have a reasonable expectation of success for the use of multitarget constructs employing the double stranded RNA molecules of Fire. Therefore, the invention is nonobvious over the disclosures of the references cited by the Examiner.

The present invention also claims expression vectors encoding multiple double stranded RNA sequences that are expressed from different promoters. This general embodiment of the invention is also nonobvious over the cited references. The Examiner dismisses the evidence provided in the declaration by Dr. McCallus that one of skill in the art would not be motivated to express different double stranded RNA sequences from separate promoters on a single vector due to the well known problem of promoter interference. The Examiner focuses on the Hull reference, which Dr. McCallus cited in his declaration as evidence that promoter interference can occur with pol III promoters as well as other types of promoters. She alleges that Hull does not teach that there is a disadvantage to using more than one promoter in a vector to express multiple nucleic acid sequences.

First, the Examiner has entirely disregarded the statements made by Dr. McCallus with respect to the widely reported phenomenon of promoter interference. See

paragraphs 6 & 7 in McCallus declaration submitted with response of February 6, 2008. The Examiner has provided no evidence to the contrary demonstrating that promoter interference is not a common problem when expressing multiple nucleic acids from a single vector.

Second, Hull discloses that the pol III promoters can interfere with nearby pol II promoters. In the introduction, Hull explains that "In some cases, cryptic pol III promoter elements directly interfere with factor binding sites in the pol II promoter upstream region or with the pol II initiation site itself." See left-hand column on page 1266. The authors then conclude that "it would not be surprising to find that the pol III promoters contribute to transcriptional regulation of the surrounding chromatin in many different contexts." See right-hand column on page 1273. The description of the problem of promoter interference provided in Dr. McCallus' declaration combined with the disclosure of Hull showing that promoter interference can also occur with pol III promoters suggest that a skilled artisan would have some doubts as to the success of efficient expression of multiple double stranded RNA molecules from different promoters on a single vector.

Furthermore, Applicants note that the disclosure of Heifetz does not teach the expression of multiple double stranded RNA molecules from different promoters, but rather a sense and an antisense strand of a *single* double stranded RNA molecule in plants. Thus, one cannot infer from the Heifetz disclosure that a vector encoding *multiple* double stranded RNA molecules under the control of different promoters would be successful in mammals. In addition, Calabretta merely suggests the possibility of expressing the two different antisense sequences from different promoters on a single

vector. In light of the literature describing the problems encountered with promoter interference, it is not apparent that a construct suggested by Calabretta would be successful or that the skilled artisan would have had any expectation of success with such a construct.

In conclusion, the skilled artisan would not have been motivated to produce long multitarget double stranded RNA molecules that target mammalian genes given the disclosures of Werther, Fire, Heifetz, and Calabretta *et al.* (US 5,734,039). First, the skilled artisan would not have attempted to use long double stranded RNA molecules as disclosed by Fire to silence genes in mammalian cells because the presence of the PKR response in these cells would prevent sequence specific gene silencing. Second, the skilled artisan would not have been motivated to express several different double stranded RNAs from a single vector, since it was well known in the prior art that competition between promoters on a single vector could result in unequal expression of the genes under the control of those promoters. Thompson was only relied upon for teaching expression of therapeutic RNAs including ribozymes and antisense RNAs using a RNA pol III promoter. Thompson does not make up for the deficiencies of Werther, Calabretta, Fire and Heifetz with regard to teaching multitarget dsRNA molecules and vector constructs expressing multiple dsRNAs. In view of all the above remarks, reconsideration and withdrawal of the rejection under §103(a) are respectfully requested.

CONCLUSION

Applicants believe that this Reply adequately addresses all the rejections of the claims, and that the claims are now in condition for allowance.

Except for issue fees payable under 37 CFR §1.18, the commissioner is hereby authorized by this paper to charge any additional fees during the pendency of this application including fees due under 37 CFR §1.16 and 1.17 which may be required, including any required extension of time fees, or credit any overpayment to Deposit Account 50-1283. This paragraph is intended to be a **CONSTRUCTIVE PETITION FOR EXTENSION OF TIME** in accordance with 37 CFR §1.136(a)(3).

If the Examiner has any further questions relating to this Reply or to the application in general, she is respectfully requested to contact the undersigned by telephone so that allowance of the present application may be expedited.

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Respectfully submitted,
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